Preprint

https://doi.org/10.5327/DST-2177-8264-1478

DST - J bras Doenças Sex Transm 2025;37:e1478

Editorial – BJSTD – November 2025

Antibiotic Resistance in *Treponema pallidum* subsp. *pallidum*, the Syphilis Agent

Resistência a antibióticos do Treponema pallidum subsp. pallidum, o Agente da Sífilis

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Keywords: Syphilis, *Treponema pallidum*, Antibiotic Resistance, Macrolides, β-lactams, Doxycycline, Penicillin Binding Protein.

Conflict of interest statement: Dr. Lorenzo Giacani declares no conflict of interest.

Syphilis, caused by *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), remains a global health problem, with a rising incidence worldwide (1, 2) and significant challenges in disease control in both

low- and middle-income countries and in high-income nations. Once syphilis is diagnosed, the recommended treatment is penicillin G in different formulations and dosages, depending on disease stage and involvement of the central nervous system (CNS) (3). In addition to penicillin G, other natural or synthetic β-lactams have shown efficacy against *T. pallidum* in pre-clinical (4) and clinical studies. Amoxicillin, for example, was effective for syphilis treatment (5, 6). Ceftriaxone, a third-generation β-lactam cephalosporin, is listed as an alternative to penicillin G for early syphilis, and data support its efficacy for neurosyphilis as well (7-9). Promising results are also available on the use of another third-generation cephalosporin, cefixime, to treat early syphilis (10, 11).

The reliance on β-lactam antibiotics for syphilis treatment is strongly justified by the low minimal inhibitory concentration (MIC) exhibited by these antibiotics when tested against *T. pallidum* (4, 12) and, most importantly, by the lack of evidence that genetic resistance ever developed to this class of antibiotics during the eighty years of uninterrupted use to treat syphilis. One possible reason why *T. pallidum* has not developed genetic resistance to β-lactams is to be found in the lack of plasmids or other mobile elements in the pathogen's genome, which makes the acquisition and retention of episomes containing genes coding for β-lactamases or other resistance genes quite unlikely. However, because *T. pallidum* is naturally competent for transformation (13), the uptake of environmental DNA fragments carrying β-lactamase genes could occur. In this scenario, one could speculate that these exogenous DNA fragments lacked sufficient homology to *T. pallidum* DNA to recombine with and integrate into the chromosome. Additionally, even if these recombination events occurred, their outcomes might not be advantageous to the pathogen, as a significant proportion of its genes are likely to be essential. During its evolution to become an obligate human pathogen, *T. pallidum* has undergone genomic reduction (14) that led to the elimination of genes encoding many metabolic and biosynthetic pathways whose products are now provided by the infected host, while retaining other essential genes and virulence factors. One could postulate that altering the sequence or expression pattern of many *T.*

pallidum genes in its minimal genome may result in a non-viable phenotype, which limits the acquisition of new genes and, hence, explains the monomorphic nature of the *T. pallidum* genome.

Regarding susceptibility to β-lactams, investigators have postulated that point-mutations in *T. pallidum* genes encoding penicillin binding proteins (PBPs) could confer resistance or limit the efficacy of these compounds. This mechanism would bypass the need for environmental DNA acquisition and risky genomic rearrangements. An early study identified five single-nucleotide polymorphisms in three penicillin-binding proteins, and the authors hypothesized that these changes could translate into structural modifications able to reduce the pathogen's susceptibility to β-lactams (15). The study, however, suffered from the unavailability of viable strains to assess the contributions of these polymorphisms *in vivo* or *in vitro*. Another study reported that one of the *T. pallidum* PBPs, the immunodominant 47 KDa lipoprotein (TpN47), exhibits β-lactamase activity *in vitro* (16). However, the authors also showed that TpN47 activity was inhibited by hydrolyzed penicillin, explaining why the syphilis agent remains susceptible to penicillin. The authors, nonetheless, hypothesized that mutations capable of bypassing this product inhibition phenomenon could arise, although no experimental evidence for those is currently reported.

In 2025, two laboratory-derived mutant *T. pallidum* strains carrying the A1873G mutation (inducing the amino acid change M625V) in the PBP-encoding *tp0705* gene were reported to be less susceptible to ceftriaxone and penicillin G at low concentrations of these antibiotics (13), although with very modest absolute effect sizes. We recently tested the *in vitro* susceptibility to penicillin G and ceftriaxone of three *T. pallidum* clinical isolates (UW244B, UW249B, and UW330B) each carrying a distinct variant of the Tp0705 protein, including the one studied in (13), due to polymorphisms at positions 1516, 1873, and 2122 of the gene. The goal of this study was to assess whether these naturally occurring Tp0705 variants would alter susceptibility to β-lactams in these strains, which have identical PBPs, except for Tp0705. These *T. pallidum* isolates, however, were all equally susceptible to penicillin G and ceftriaxone (Tantalo *et al.*, submitted, STD).

Overall, even in the absence of concrete evidence for resistance to β-lactams in the syphilis agent, one should assume that resistance could eventually emerge. Although reports of penicillin treatment failures remain relatively rare, investigators should do their best to obtain treponema-containing samples from patients who allegedly failed β-lactam treatment for further studies. At the same time, given that low concentrations of β-lactams are not treponemicidal, an effort should be made to determine the post-administration level of these antibiotics attain in tissues and bodily compartments known to harbor *T. pallidum* during infection, so that adequate concentrations can be achieved and maintained to ensure cure.

In contrast to β-lactams, resistance to macrolides developed and spread rapidly when oral azithromycin was introduced for early syphilis treatment, to the point that virtually all *T. pallidum* strains circulating in North America are macrolide-resistant (17). In this pathogen, macrolide resistance is associated with either the A2058G or the A2059G mutations in the 23S rRNA gene (18). Currently, based on available molecular typing data, the percentage of macrolide-resistant *T. pallidum* strains varies by country and is likely to reflect the extent of macrolide use within each healthcare system. Even in countries where such a percentage is not near 100%, providers should avoid prescribing azithromycin for early syphilis and assume that their patients are infected with a macrolide-resistant strain to minimize the risk of treatment failure, disease progression, and selection of these resistance-associated mutations.

Doxycycline and tetracycline have been used for years and are effective for early and latent syphilis (3). Whether *T. pallidum* can develop resistance to doxycycline and tetracyclines is also the topic of an ongoing debate. Tetracyclines block protein synthesis by binding to the 16S rRNA within the 30S ribosomal subunit (19). Past studies demonstrated that doxycycline had a success rate comparable to penicillin for early syphilis (20-23). In other bacterial pathogens, tetracycline or doxycycline resistance can be mediated by the acquisition and expression of efflux systems, ribosomal protection proteins, or drug-inactivating enzymes (19). In a recent report, authors claimed to have identified the efflux pump-

encoding gene *tetB* in 15 samples from syphilitic lesions (24); however, the detected resistance gene might also have been from other bacteria also present in the sampled area. Mutations in ribosomal protein-encoding genes or in the 965-967 triplet of the 16S rRNA gene have also been associated with tetracycline resistance in other pathogens (19). Whether those changes would confer tetracycline resistance if present in the *T. pallidum* 16S rRNA gene is unclear.

While there are no reliable reports of a tetracycline-resistant *T. pallidum* strain, concerns persist regarding the possible emergence of doxycycline resistance following the widespread adoption of doxycycline post-exposure prophylaxis (doxy-PEP). The increased and intermittent use of doxycycline among individuals at risk for STIs could favor selection of doxycycline resistance in *T. pallidum* in the same way macrolide use selected for resistance-associated mutations in the syphilis agent. In a recent *in vitro* study we authored, *T. pallidum* was intermittently exposed to doxycycline for over seven months and then continuously for ten weeks. Following these prolonged exposures, genotypic and phenotypic analyses found no doxycycline resistance, although the tested strain appeared to exhibit some limited tolerance to low concentrations of doxycycline toward the end of the exposure experiment, which, however, did not result in any change in the drug's minimal inhibitory concentration (MIC) for *T. pallidum* (25).

In conclusion, although *bona fide* resistance to antibiotics in *T. pallidum* has been described so far only for macrolides, it has never for other classes of antibiotics used to treat syphilis. Given the foreseeable increase in the use of prophylactic doxycycline for sexually transmitted bacterial infection and the fact that approved therapeutics for syphilis remain limited, heightened surveillance and alert will be pivotal for this pathogen in the years to come. Thanks to advances in *in vitro* propagation of *T. pallidum* (4) and a deeper understanding of *T. pallidum* genomics (26), research into alternative effective medications for syphilis is ongoing and will likely lead to new treatment options in the future, which will further mitigate the threat of antibiotic resistance in this pathogen.

ACKNOWLEDGEMENTS

The writing of this manuscript was supported by NIH Grant R56AI175016 (to L.G.). The funders of the study had no role in the study design, data collection, data analysis, data interpretation, manuscript writing, or in the decision to submit it for publication.

REFERENCES

1. WHO. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021 - Accountability for the global health sector strategies 2016–2021: actions for impact. https://wwwwhoint/publications/i/item/9789240027077. 2021.
2. WHO. World Health Organization. Syphilis. 2024. https://wwwwhoint/news-room/fact-sheets/detail/syphilis. 2024.
3. CDC. STD Treatment Guidelines - Syphilis. Morbidity and Mortality Weekly Report. 2021.
4. Tantalo LC, Lieberman NAP, Pérez-Mañá C, Suñer C, Vall Mayans M, Ubals M, et al. Antimicrobial susceptibility of Treponema pallidum subspecies pallidum: an in-vitro study. Lancet Microbe. 2023;4(12):e994-e1004.
5. Nishijima T, Kawana K, Fukasawa I, Ishikawa N, Taylor MM, Mikamo H, et al. Effectiveness and Tolerability of Oral Amoxicillin in Pregnant Women with Active Syphilis, Japan, 2010-2018. Emerg Infect Dis. 2020;26(6):1192-200.
6. Tanizaki R, Nishijima T, Aoki T, Teruya K, Kikuchi Y, Oka S, et al. High-dose oral amoxicillin plus probenecid is highly effective for syphilis in patients with HIV infection. Clin Infect Dis. 2015;61(2):177-83.
7. Shann S, Wilson J. Treatment of neurosyphilis with ceftriaxone. Sex Transm Infect. 2003;79(5):415-6.

8. Cao Y, Su X, Wang Q, Xue H, Zhu X, Zhang C, et al. A Multicenter Study Evaluating Ceftriaxone and Benzathine Penicillin G as Treatment Agents for Early Syphilis in Jiangsu, China. Clin Infect Dis. 2017;65(10):1683-8.

9. Marra CM, Boutin P, McArthur JC, Hurwitz S, Simpson PA, Haslett JA, et al. A pilot study evaluating ceftriaxone and penicillin G as treatment agents for neurosyphilis in human immunodeficiency virus-infected individuals. Clin Infect Dis. 2000;30(3):540-4.

10. Stafylis C, Keith K, Mehta S, Tellalian D, Burian P, Millner C, et al. Clinical Efficacy of Cefixime for the Treatment of Early Syphilis. Clin Infect Dis. 2021;73(5):907-10.

11. Klementová T, Zákoucká H, Bížová B, Unemo M, Rob F. Cefixime versus benzathine penicillin G for the treatment of early syphilis-a randomized, controlled open label trial. J Antimicrob Chemother. 2025.

12. Hayes KA, Dressler JM, Norris SJ, Edmondson DG, Jutras BL. A large screen identifies beta-lactam antibiotics which can be repurposed to target the syphilis agent. NPJ Antimicrob Resist. 2023;1(1):4.

13. Pospíšilová P, Bosák J, Hrala M, Krbková L, Vrbová E, Šmajs D. Resistance to ceftriaxone and penicillin G among contemporary syphilis strains confirmed by natural *in vitro* mutagenesis. Commun Med (Lond). 2025;5(1):224.

14. Smajs D, Norris SJ, Weinstock GM. Genetic diversity in *Treponema pallidum*: Implications for pathogenesis, evolution and molecular diagnostics of syphilis and yaws. Infect Genet Evol. 2012;12(2):191-202.

15. Sun J, Meng Z, Wu K, Liu B, Zhang S, Liu Y, et al. Tracing the origin of *Treponema pallidum* in China using next-generation sequencing. Oncotarget. 2016;7(28):42904-18.

16. Cha JY, Ishiwata A, Mobashery S. A novel beta-lactamase activity from a penicillin-binding protein of *Treponema pallidum* and why syphilis is still treatable with penicillin. J Biol Chem. 2004;279(15):14917-21.

17. Lieberman NAP, Reid TB, Cannon CA, Nunley BE, Berzkalns A, Cohen SE, et al. Near-Universal Resistance to Macrolides of *Treponema pallidum* in North America. N Engl J Med. 2024;390(22):2127-8.

18. Stamm LV. Global challenge of antibiotic-resistant *Treponema pallidum*. Antimicrob Agents Chemother. 2009;54(2):583-9.

19. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Microbiol Spectr. 2016;4(2).

20. Wong T, Singh AE, De P. Primary syphilis: serological treatment response to doxycycline/tetracycline versus benzathine penicillin. Am J Med. 2008;121(10):903-8.

21. Ghanem KG, Erbelding EJ, Cheng WW, Rompalo AM. Doxycycline compared with benzathine penicillin for the treatment of early syphilis. Clin Infect Dis. 2006;42(6):e45-9.

22. Harshan V, Jayakumar W. Doxycycline in early syphilis: a long term follow up. Indian J Dermatol. 1982;27(4):119-24.

23. Onoda Y. [Therapeutic effect of oral doxycycline on syphilis (author's transl]. Jpn J Antibiot. 1980;33(1):18-28.

24. Xiao H, Li Z, Li F, Wen J, Liu D, Du W, et al. Preliminary study of tetracycline resistance genes in Treponema pallidum. J Glob Antimicrob Resist. 2017;9:1-2.

25. Tantalo LC, Luetkemeyer A, Lieberman NAP, Nunley BE, Avendaño C, Greninger AL, et al. Long-term in vitro exposure of *Treponema pallidum* to sub-bactericidal doxycycline did not induce resistance: Implications for doxy-PEP and syphilis. J Infect Dis. 2024.

26. Lieberman NAP, Lin MJ, Xie H, Shrestha L, Nguyen T, Huang ML, et al. *Treponema pallidum* genome sequencing from six continents reveals variability in vaccine candidate genes and dominance of Nichols clade strains in Madagascar. PLoS Negl Trop Dis. 2021;15(12):e0010063.